REMARKS

Applicants thank the Examiner for the interesting telephone interview on February 23, 2010. As discussed in the interview, the phrase "nuclease gene product" has been amended to clarify that it refers solely to the enzyme, not the nucleic acid molecule encoded by the gene.

The claims have also been amended to require the bacteria produce polyhydroxyalkanoates ("PHAs") - naturally or are genetically engineered, rather than just be suitable for the production of PHAs. Support for this amendment is found in the application, for example, at page 16, lines 15-17, and example 6.

In response to the Advisory Action, the claims have been clarified to require:

(1) expression of a nuclease

And

(2) secretation of the nuclease into the periplasmic space

(3) in an amount effective to degrade at least 95% of all of the nucleic acid released following lysis of the cells in less than 24 hours.

Rejection Under 35 U.S.C. § 102

Claims 1, 2, 4, 6, and 8 were rejected under 35 U.S.C. § 102(b) as anticipated by Liebl, *et al.*, *J. Bacteriology* 174(6):1854-1861 (1992) ("Liebl"). Applicants respectfully traverse this rejection.

The Legal Standard

For a rejection of claims to be properly founded under 35 U.S.C. § 102, it must be established that a prior art reference discloses each and every element of the claims. *Hybritech*

Inc. v. Monoclonal Antibodies Inc., 231 U.S.P.Q. 81 (Fed. Cir. 1986); *Scripps Clinic & Research Found. v. Genentech Inc.*, 18 U.S.P.Q.2d 1001 (Fed. Cir. 1991), and it must enable a person skilled in the art to make and use the invention. "A claimed invention cannot be anticipated by a prior art reference if the allegedly anticipatory disclosures cited as prior art are not enabled". *Amgen, Inc. v. Hoechst Marion Roussel, Inc.*, 314 F.3d 1313, 1354, 65 U.S.P.Q.2d 1385, 1416 (Fed. Cir. 2003).

Despite any ongoing disagreement over the proof needed for inherency, there is a general agreement in the case law about the basic requirement for something to be considered "inherent". This requirement is that there must be a "necessary" causal relation between the disclosed features and the omitted, allegedly inherent one(s):

> It is well settled that a prior art reference may anticipate when the claim limitations not expressly found in that reference are nonetheless inherent in it. Under the principles of inherency, if the prior art necessarily functions in accordance with, or includes, the claimed limitations, it anticipates.

In re Cruciferous Sprout Litig., 301 F.3d 1343, 1349 (Fed.Cir.2002). Stated negatively, the courts have consistently held that proof of inherency is not met by a mere showing of a possibility or probability that the missing element or function is present. *Trintec Indus., Inc. v. Top-U.S.A. Corp.*, 295 F.3d 1292, 1295 (Fed.Cir.2002); *Continental Can Co. v. Monsanto Co.*, 948 F.2d 1264, 1269 (Fed.Cir.1991).

"To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is **necessarily present in the thing described in the reference, and that it would be so recognized by persons** of ordinary skill. Inherency, however, may not be

established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.' " *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999) (citations omitted). "In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art." *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990) (emphasis in original).

Analysis

Liebl does not anticipate the claimed subject matter. The bacterial strain of Liebl is not genetically engineered to produce PHAs, nor does it produce PHAs. Therefore, Liebl does not disclose each feature of the claimed bacterial strain.

Liebl also does not anticipate under the doctrine of inherency. There is no disclosure that *C. glutamicum* produces PHAs, that it is genetically engineered to produce PHAs, nor that it expresses nor secretes into the periplasmic space the claimed amount of nuclease. As demonstrated by the examples in the application, of the 50 nuclease positive bacterial strains expressing nuclease, only two out of 50 expressed high levels. See page 15. This demonstrates that one skilled in the art would not be able to predict that the strain of Liebl, IF it produced PHA, and IF it were expressing nuclease that is secreted into the periplasm, that it would necessarily be in an amount as defined by claim 1.

Rejection Under 35 U.S.C. § 103

Claims 1-4 and 6-8 were rejected under 35 U.S.C. § 103(a) as obvious over WO 94/10289 by Greer, *et al.*, ("Greer"), Atkinson, *et al.*, Biochemical Engineering and Biotechnology Handbook, 2nd Edition, Stockton Press: New York, 1991 ("Atkinson") and Lee, *et al.*, *Adv. Biochem. Eng. Biotechnol.* 52:27-58 (1995) ("Lee"), or Miller, *et al.*, *J. Bacteriology* 169(8):3508-3514 (1987) ("Miller") in view of Liebl et al., *J. Bacteriology* 174(6):1854-1861 (1992) ("Liebl"), or Miller. Applicants respectfully traverse this rejection.

The Legal Standard

When applying 35 U.S.C. § 103, the following tenets of patent law must be adhered to:

(a) determining the scope and contents of the prior art;

(b) ascertaining the differences between the prior art and the claims in issue;

(c) resolving the level of ordinary skill in the pertinent art; and

(d) evaluating evidence of secondary consideration.

Graham v. John Deere, 383 U.S. 1, 17-18, 148 U.S.P.Q. 459, 467 (1966). These four factors are traditionally referred to as the Graham factors.

Obviousness is a legal conclusion. *See Graham v. John Deere Co.*, 383 U.S. 1, 17-18, 148 U.S.P.Q. 459 (1966). The *Graham* analysis was recently affirmed by the Supreme Court in *KSR Int'l Co. v. Teleflex, Inc.*, 127 S. Ct. 1727, 82 U.S.P.Q.2d 1385 (2007).

The obviousness analysis requires looking at the invention as a whole. "Focusing on the obviousness of substitutions and differences, instead of on the invention as a whole, is a legally improper way to simplify the often difficult determination of obviousness." *Gillette Co. v. S.C.*

Johnson & Sons, Inc., 919 F.2d 720, 724, 16 U.S.P.Q.2d 1923 (Fed. Cir. 1990); *see Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1383, 231 U.S.P.Q. 81, 93 (Fed. Cir. 1986).

Hindsight analysis, such as picking and choosing from prior art references using the claimed invention as a template, has long been forbidden. *See, e.g., In re Fine*, 837 F.2d 1071, 1075 (Fed. Cir. 1988), which states that "One cannot use hindsight reconstruction to pick and choose among isolated disclosures on the prior art to deprecate the claimed invention." In *KSR*, the Court also warned against the use of hindsight analysis in making an obviousness determination. The Court stated, "A factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon *ex post* reasoning." (*KSR*, 127 S. Ct. at 1742, citing *Graham*, 383 U.S. at 36 (warning against a "temptation to read into the prior art the teachings of the invention in issue" and instructing courts to "guard against slipping into the use of hindsight" (quoting M*onroe Auto Equipment Co. v. Heckethorn Mfg. & Supply Co.*, 332 F.2d 406, 412, 141 U.S.P.Q. 549 (6th Cir. 1964))).

Analysis

The Scope and Content of the Prior Art

Greer

Greer describes the exogenous addition of peroxide to a cell culture. As stated in the Examples of Greer, and as stated as one of the problems addressed by the presently claimed invention, the exogenous addition of nucleases is generally known and too expensive to use for commodity fermentation products involving high cell density fermentations.

Liebl

Liebl describes the heterologous expression of a *Staphylococcus aureus* nuclease gene in *C. glutamicum* and the use of this transgenic system for investigating protein export in *C. glutamicum*, as discussed above. Liebl does not express the nuclease in any amount, much less that which is claimed functionally, nor does it disclose bacteria which naturally produces PHA nor which have been genetically engineered to produce PHAs.

Miller

Miller discloses the use of a *B. subtilis* secreted nuclease for investigating "the nature of the processing of the nuclease signal peptide". Miller further characterizes the secretion of nuclease and the processing of the signal peptide from the precursor protein in *B. subtilis*. Miller speculates that the *staphylococcal* nuclease and its gene may be very useful for the development of secretion vectors for foreign proteins.

Atkinson

Atkinson is a general review of biochemical and biotechnological methods and reagents.

Lee

Lee reports on production of PHAs in bacteria, and control of fermentation conditions.

The Differences Between the Prior Art and the Claims

A combination of Greer, Liebl, Miller, Atkinson and Lee does not recite all of the elements of the claims.

None of the references cited by the Examiner disclose or suggest the claimed bacterial strain. None of the references cited by the Examiner disclose expression and secretion of a

nuclease into the periplasmic space of any gram negative bacteria, in any amount (see Liebel declaration filed December 10, 2009), nor production of PHAs.

None of the references disclose the problem to be solved except for Greer, which teaches away from the claimed solution to this long standing problem, by advising those skilled in the art to add exogenous nuclease to the lysate. Applicants provide an elegant system for reducing viscosity in fermentation media whereby bacteria are engineered to express an effective amount of nuclease, which is sequestered in the periplasmic space where it is harmless to the cells, until it is released when needed, by cell lysis. There is nothing in any of the references cited by the Examiner which would lead one of ordinary skill to this limitation.

Liebl does not disclose expression of the SNase gene product in *E. coli.* Liebl discloses propagation of a plasmid containing the SNase gene. Thus if the *E. coli* in Liebl were lysed, the plasmid would be released, not the nuclease. There is no basis for the assertion that cell lysis will lead to secretion of the gene product into the periplasmic space. With respect to the *C. glutamicum* disclosed in Liebl, is it unclear how the bacteria which are engineered to secrete nuclease into cell culture medium would secrete the nuclease into the periplasmic space if lysed. *C. glutamicum* does not have a periplasmic space. *See* Zuber, *J. Bacteriol.*, 190(16):5672-5680 (2008) (a copy of which was previously submitted December 10, 2009). See especially, Fig. Legend of Fig.1).

None of Atkinson or Lee makes up for the deficiencies in Greer, Liebl and Miller. Lee discloses the production of copolyesters (not PHAs) in *Pseudomonas sp.* Lee does not disclose genetically modifying any bacteria for secretion of nuclease into the periplasmic space.

Atkinson, a review of biochemical and biotechnological methods and reagents, similarly does not make up for this deficiency.

With respect to claim 7, none of the prior art discloses genetically modifying bacteria with the heterologous nuclease gene integrated into the chromosome, and the gene product secreted into the periplasmic space.

The cited art, alone or in combination does not provide an expectation of success in arriving at the claimed bacterial strains

At time of publication of Liebl or Miller, it was widely believed that gram positive bacteria did not have a periplasmic space (see Sakamoto, *et al.*, *Microbiology*, 147:2865-2871 (2001), submitted by Applicants with the amendment and response filed on October 24, 2007). Thus, neither Liebl nor Miller both of whom expressed genes in gram positive bacteria, were concerned with targeting genes into the periplasmic space. Therefore, contrary to the Examiner's allegations, neither Miller nor Liebl provides one with an expectation of success in expressing a nuclease in the periplasmic space as claimed. Office Action mailed March 12, 2009, page 9. Atkinson, a review of biochemical and biotechnological methods and reagents, similarly does not make up for this deficiency. Thus, a combination of references, all of which are concerned with addressing a different technical problem, does not provide one with an expectation of success in expressing a nuclease gene product in the periplasmic space as claimed, which is released upon lysis of the bacteria.

Evidence of secondary considerations

As the Court reiterated in *KSR v. Teleflex*, evidence of long standing need and of commercial success are both secondary indicia of non-obviousness. Secondary considerations to be considered include commercial success, long felt but unresolved needs, failure of others, unexpected results, properties not present in the prior art etc.

The claims provide a simple yet elegant mechanism to endogenously produce nuclease in genetically modified bacteria, which enables cost effective processing of nucleic acids released during fermentation processes, avoids any deleterious effects of the expressed nuclease on the cells, and provides external control of the release of the nuclease. This is accomplished by directing expression of the nuclease to the periplasmic space. Such expression sequesters the nuclease, protecting the cells from the nuclease until needed, and enables release of nuclease into the fermentation mediums when desired. None of the references cited by the Examiner, alone or in combination provide bacteria with the properties of the claimed bacteria.

With respect to claim 7, chromosomal integration of the heterologous nuclease and expression of nuclease to high levels (shown in Example 6) avoids the use of plasmids which are difficult and expensive to maintain in large scale fermentations, as well as the use of IPTG which is cost prohibitive and toxic (see Makrides, page 514, left col.) that has been necessary in plasmid-based expression systems in the prior art, to obtain appreciable expression/secretion of nuclease. Such cost effective strains are highly desirable. The claims provide microbial strains which are cost effective for fermentation processes and can enable more profitable production of polyhydroxyalknoates.

The prior art, alone or in combination, does not provide the claimed bacterial strain. Accordingly, claims 1, 3, 4, and 6-9 are not obvious over Greer, Aktinson, and Lee or Miller in view of Liebl or Miller.

Allowance of claims 1, 3, 4, 6-9, 11, 12, 14-16, and 19 is respectfully solicited. Claims 11, 12, 14-16 and 19 are related to claims 1, 3, 4, 6-9 and 14-16 as product and process of use. Accordingly, no new search would be required should claims 1, 3, 4, and 6-9 be found to be allowable.

Respectfully submitted,

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Date: May 3, 2010

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